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Original Contribution

Role of mtDNA haplogroups in COPD susceptibility in a southwestern Han Chinese population

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ABSTRACT

The interplay of a complex genetic basis with the environmental factors of chronic obstructive pulmonary disease (COPD) may account for the differences in individual susceptibility to COPD. Mitochondrial DNA (mtDNA) contributes to an individual's ability to resist oxidation, an important determinant that affects COPD susceptibility. To investigate whether mtDNA haplogroups play important roles in COPD susceptibility, the frequencies of mtDNA haplogroups and an 822-bp mtDNA deletion in 671 COPD patients and 724 control individuals from southwestern China were compared. Multivariate logistic regression analysis revealed that, whereas mtDNA haplogroups A and M7 might be associated with an increased risk for COPD ($OR=1.996$, 95% $CI=1.149–2.831$, $p=0.006$, and $OR=1.754$, 95% $CI=1.931–2.552$, $p=0.021$, respectively), haplogroups F, D, and M9 might be associated with a decreased risk for COPD in this population ($OR=0.554$, 95% $CI=0.390–0.787$, $p=0.001$; $OR=0.758$, 95% $CI=0.407–0.965$, $p=0.002$; and $OR=0.186$, 95% $CI=0.039–0.881$, $p=0.034$, respectively). Additionally, the increased frequency of the 822-bp mtDNA deletion in male cigarette-smoking subjects among COPD patients and controls of haplogroup D indicated that haplogroup D might increase an individual's susceptibility to DNA damage from external reactive oxygen species derived from heavy cigarette smoking. We conclude that haplogroups A and M7 might be risk factors for COPD, whereas haplogroups D, F, and M9 might decrease the COPD risk in this Han Chinese population.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a reduction in lung function with airflow obstruction that is not fully reversible. The airflow restriction is usually progressive and is associated with an abnormal lung inflammatory response directed against noxious particles or gases [1]. COPD is a leading cause of morbidity and mortality worldwide and is predicted to become the fourth leading cause of death in the United States by the year 2030 [2]. COPD is also a common health problem in China. Epidemiological studies indicate that COPD is the leading cause of mortality in rural areas and the fourth leading cause of death in urban areas in China [3]. The World Health Organization predicts that COPD will become the leading financial burden on health care systems in China by the year 2020.

Cigarette smoking is the principle environmental risk factor for the development of COPD. However, it is well known that susceptibility to COPD also has a complex genetic basis that interacts with environmental factors and that these interactions may account for the differences in individual susceptibility [4]. Over the past several years, a variety of approaches, including candidate-gene association studies, linkage analysis, and rare-variant studies, have been utilized to search for COPD susceptibility loci. Although many candidate loci have been identified, varying degrees of reproducibility have been observed due to small sample sizes [5–7]. Remarkably, genome-wide association studies (GWAS) of COPD with relatively larger sample sizes have provided compelling evidence for COPD susceptibility loci on chromosomes 4q22 [8], 4q31 [9,10], 15q25 [9], and 19q13 [6], and, to date, three susceptibility loci, *CHRNA3/CHRNA5/IREB2* (15q25), *HHIP* (4q31), and *FAM13A* (4q22), have been well replicated [8,9,11–19]. Recently, the increased transcription factor Sp3 binding at a functional single-nucleotide polymorphism of *HHIP*, a COPD GWAS locus on 4q31, has been found to lead to reduced *HHIP* expression and increased susceptibility to COPD through distal transcriptional regulation, which provided functional interpretations of the COPD susceptibility loci in the pathogenesis of COPD [20]. It was worth following closely that among the COPD susceptibility loci identified by GWAS [6,8–10],

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several loci have also been found to be associated with lung cancer risk [21,22], nicotine metabolism [23–25], or hypoxia [26,27]. The findings gave us the clue that mitochondrial DNA (mtDNA) variants might also be associated with COPD susceptibility.

The reactive oxygen species (ROS) present in cigarette smoke are thought to contribute to the development and progression of COPD through the induction of systemic oxidative stress, which can result in tissue injury, muscle dysfunction, organ failure, and sustained systemic inflammation. Although the gaseous-phase ROS in cigarette smoke are not capable of diffusing through the plasma membranes of airway epithelial cells and are thus unlikely to pass through the airway epithelial cell barrier, the lipophilic components in cigarette smoke, including phenolic compounds, aldehydes, and polycyclic aromatic hydrocarbons, can easily penetrate airway epithelial cells and enter the systemic circulation. Once in the circulation, the lipophilic components can disturb mitochondrial function, leading to the increased intracellular generation of mitochondrial ROS [28,29]. The potential importance of mitochondrial ROS metabolism in COPD is best illustrated by the fact that many antioxidants important in COPD pathophysiology are linked to mitochondria, including glutathione, thioredoxin, superoxide dismutase, and heme oxygenase-1 [30].

Mitochondria are present in all eukaryotic cells and use oxidative phosphorylation (OXPHOS) to convert the chemical energy of carbohydrates and fats into usable energy forms. Mitochondria also generate approximately 85% of intracellular ROS, which can promote cellular differentiation or induce apoptosis [31,32]. Unlike any other cellular organelle, mitochondria have their own DNA. Human mtDNA is a circular molecule approximately 16.5 kb in size; it encodes 22 transfer RNAs (tRNAs), 2 ribosomal RNAs, and 13 respiratory chain subunits that are essential for mitochondrial respiratory functions [31]. MtDNA is particularly susceptible to oxidative damage and mutation because of the high rate of ROS production and the limited DNA-repair capacity in mitochondria [33]. Cigarette smoking is an environmental factor that induces oxidative stress by creating high ROS levels within the body [34–36]. Chronic oxidative stress can induce mtDNA damage, resulting in point mutations, insertions, and deletions [37]. The accumulation of oxidative damage and the resulting sequence variations in mtDNA can ultimately lead to abnormal OXPHOS in affected cells [38]. As such, the oxidative stress induced by cigarette smoking may play a substantial role in the pathogenesis of smoking-related diseases.

Interestingly, prior studies have demonstrated that the common and "nonpathological" mtDNA variations that define the various mtDNA haplogroups are associated with susceptibility to metabolic and degenerative diseases in humans; they also influence longevity and carcinogenesis under conditions where mitochondrial ROS production is thought to play a role [32,39–41]. Further, mtDNA variations defining the various mtDNA haplogroups also determine differences in OXPHOS performance and ROS production in both mice and humans [42–45]. Furthermore, the increased number of mtDNA copies that are induced to compensate for damage has been found to be positively associated with an increased risk for lung cancer among heavy smokers [46]. Additionally, in our prior study, several mtDNA haplogroups were associated with increased lung cancer risk in a Han Chinese population from southwestern China [47].

Because cigarette smoking is one of the common environmental risk factors contributing to the development of lung cancer and COPD, and lung cancer and COPD are linked at the molecular and genetic levels [8,9,11,12], we hypothesized that mtDNA haplogroups might also play a role in COPD susceptibility. To test the hypothesis, we performed a case–control study to investigate the association between mtDNA haplogroups and COPD risk in a Han Chinese population from southwestern China. In addition, the gene–environment interactions between the mtDNA haplogroups and cumulative cigarette smoking were analyzed.

Materials and methods

Study population

This study was approved by the Ethics Committee of Xinqiao Hospital, Third Military Medical University (Chongqing, China). A total of 671 COPD patients were recruited from the Institute of Human Respiratory Disease of Xinqiao Hospital, Third Military Medical University, from September 2007 to September 2009. In total, 724 sex-matched volunteer samples were collected from individuals being examined at the Center of Physical Examination at Xinqiao Hospital, Third Military Medical University, between November 2007 and September 2009. All of the subjects were unrelated for at least three generations. After the purpose and procedures of the study were explained, all participants signed a written informed consent form and completed a detailed questionnaire regarding their smoking habits. For all subjects, blood samples were drawn into Na–EDTA tubes and stored at $-70\,^{\circ}C$ before genomic DNA extraction.

The COPD patients selected for this study were all initially classified as having clinically definite and laboratory-supported definite COPD; COPD staging was classified based on post-bronchodilator spirometry according to the Global Initiative for Chronic Obstructive Lung Disease (http://www.goldcopd.org/2011) criteria. Patients were excluded from the study if they had established diagnoses of asthma, cystic fibrosis, tuberculosis, lung cancer, cardiac disease, obesity, or diabetes as determined through clinical and radiographic examinations. The exclusion criteria for the control group were any history of chronic lung disease or atopy, an acute pulmonary infection in the 4 weeks preceding study assessment, or a family history of COPD.

MtDNA haplogrouping

Genomic DNA was extracted from whole blood using the QIAamp DNA blood mini kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). MtDNA haplogrouping was completed as described by Li and colleagues [47,48]. Briefly, the entire mtDNA sample was amplified into 22 overlapping PCR fragments and then digested with 14 restriction endonucleases [49,50]. A reaction without template DNA was included in each PCR–restriction fragment length polymorphism (PCR-RFLP) analysis for mtDNA haplogrouping to avoid artificial contamination caused by potential sample crossover. PCR-RFLP analysis was supplemented by sequencing of hypervariable segment I (HVS I) from position 16024 to 16383, relative to the revised Cambridge reference sequence (rCRS) of mtDNA [51]. The mtDNA haplotypes determined based on the PCR-RFLP analysis and HVS I sequences were classified into haplogroups according to mtDNA phylogenetic analysis performed using Mitomap-Phylogeny [52–54].

Detection of the 822-Bp deletion in mtDNA

Detection of the 822-bp mtDNA deletion in samples was completed as described by Zheng et al. [47]. In brief, PCR amplification using primer pair mtDNA-1 (forward, 5′–TTCG-CCTACACAATTCTCCG-3′) and mtDNA-2 (reverse, 5′–ACAGATACT-GCGACATAGGG-3′) was performed in a total volume of 25 μl. The